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A study to determine the effectiveness of dietary supplementation in pediatric patients with nutritional and health concerns

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***Abstract*---**Aim: The study's main objective was to see whether the well-known probiotic *Bifidobacterium lactis* might aid in the development of children. Method: A nine-week clinical study involving 400 children aged between 5 to 10 years, was conducted to evaluate the efficacy of food supplementation. Stage 1 participants took 150 ml of low-fat milk twice a day for three weeks as a baseline diet control. Probiotics (*Bifidobacterium lactis*) were added to milk during stage 2 in a usual dosage (4×10^{10} organisms /d) or a low dose (4×10^{9} organisms /d) for 3 weeks. During stage 3, for three weeks, they took low-fat milk. Peripheral blood samples were used to examine changes in the ratios of tumor-cell-killing and leukocyte phagocytic activities. Result: The level of NK cells and T lymphocytes were found to be increased in the blood of the individuals. NK cell's tumoricidal activity was also increased following *B. lactis* intake, as were the phagocytic capacities of mononuclear and polymorphonuclear phagocytes. Subjects with weak pretreatment immune responses had the highest improvement in immunity. Conclusion: Supplementing the diets of children with *B. lactis* (a probiotic) may help improve their cellular immunity and physical development.

Keywords*---B. lactis*, Bone density, leukocyte, Probiotics, Pediatric.

Introduction

As many as 40% of children under the age of 5 are believed to be stunted in poor nations [1].[1] For years, researchers debated whether stunting is an adaptive

response to low nutritional intakes or has functional repercussions.[2] Several cross-sectional observational studies have demonstrated that stunting is related to poor development in early children [3,4] and low school performance or IQ levels in later children.[5-7] The challenges that stunted children face might also have a negative impact on their development.[8] Observational studies are unlikely to be able to account for all of these variables.[9] A substandard diet may also affect growth,[10] as may the quality of the stimuli provided. There are design flaws in trials of nutritional supplements provided to pregnant women and their children,[11] such as the absence of random assignment to treatment groups [12] and the lack of control for any additional attention that children may get.[13,14] Some evidence suggests that supplementing children's diets with vitamins and minerals may have a positive impact on their development.

The supplementation of already malnourished children has only been studied once, and that research included developmental assessments. The children's growth was not aided by supplementation alone. As a result, it is impossible to be confident if the children's nutritional intake and development were increased. Supplementation and stimulation had a positive effect on children. However, it was impossible to differentiate the effects of stimulation alone from those of supplementation since no such group had been established. Many people across the globe spend a lot of money feeding undernourished children, although no conclusive proof exists that this helps their growth. Stunted children in particular have not been studied.[15]

Probiotic *Bifidobacterium lactis* was tested on pediatric patients in the present study to see whether it affected cellular immunity throughout a nine-week dietary intervention experiment. Human and animal studies have previously shown that this bacterium has immune-enhancing characteristics.[16,17]

Material and Method

Material

Between January 2022 and June 2022, pediatric children at Max hospital in Delhi, India, were included in this nutritional experiment. A total of 400 children were included. The phagocytosis and NK cell tests, as well as measurements of height, weight, and bone density, all had statistical power estimates of 98 percent and 92 percent, respectively. 220 males and 180 females participated in the study. All of the participants were living in their parents take care.

Interviews with the participants and consultations with their medical providers were used to determine their eligibility for the study. Children with pediatric disorders and a willingness to either follow trial rules or notify investigators if they did not comply were eligible for participation. The individual’s milk -product allergies were excluded. Subjects gave their approval after learning that the milk contain beneficial microbes. The Himgiri Zee University, Dehradun, has given its approval to the study's protocols.

Diet

The New Zealand Dairy Board provided powdered low-fat milk (LFM) in India. *B. lactis* was sourced from the Forest Research Institute's microbial strain collection (Dehradun). For the LFM powder, a typical dosage of 1×10^9 organisms/g powder was used; for the low-dose powder, 1×10^8 organisms/g powder was used. Stage 2 of the experiment was divided into four groups of 100 participants each, with one group receiving the standard dosage of probiotic supplement and the other receiving the reduced dose of probiotic supplement at random. A 25-gram sachet of low-fat milk powder or *Bifidobacterium lactis*–supplemented low-fat milk powder was vacuum-sealed within the sachets to keep the diets fresh. It was kept at 25^0 Celsius and distributed straight to the participants, who were given the packets. There was an adequate quantity of sachets available at each stage of the experiment, which was replenished at frequent intervals by the healthcare provider of each participant (every 3 week). The powder in the sachets was mixed with 150 ml of cold drinking water by the participants just before consumption, as per the instructions.

Method

The study used a three-stage, pre-, and post-intervention design to measure changes in immunity as a result of food modifications. For data analysis, each participant acted as an independent internal control. There were no supplemented milk options for the first three weeks of the study (weeks 1–3). Consuming milk fortified with *B. lactis* was done in the second stage of the experiment (weeks 4–6): The normal dosage of *Bifidobacterium lactis* (total intake: 4×10^{10} organisms/d) was ingested by 100 boys and 60 girls, whereas the low-dose group (total intake: 4×10^9 organisms/d) was consumed by 120 boys and 60 girls. All individuals received non-supplemented milk throughout the washout period (weeks 7–9) of the study. Health care providers interviewed the participants one-on-one to evaluate their overall well-being at each of the trial's immunological assessment time points and physiological measures. They were asked to indicate whether they were adhering to or deviating from a set of dietary rules.

Immune Measurements and Blood sampling

Participants gave blood at four points in the trial: Week 0 (before the trial began), week 3 (after consuming milk that was not supplemented for three weeks), week 6 (after they had consumed *Bifidobacterium lactis*–supplemented milk for three weeks), after that week 9 (after they had taken the *B. lactis*–supplemented milk for three weeks) (last three weeks of the study, individuals ceased taking *B. lactis* and drank un-supplemented milk). Subject identity and time points were included in the number coding of blood samples, but the significance of these codes was unknown to the laboratory staff. Fluorochrome-conjugated monoclonal antibodies were used to identify major leukocyte subsets in whole blood samples, such as CD3+, CD56+, CD4+, CD19+, CD8+, CD25+, and HLADR+. The proportion of mononuclear cells that stain positively for each cell surface marker is how the data are presented.

Whole-blood samples were used to test in vitro phagocytic activity after fluoresceinated *E. coli* was taken up. As a result of flow cytometry, the proportion of each leukocyte sample demonstrating phagocytic activity was determined for monocytes and polymorphonuclear cells. Mononuclear cells were isolated from the blood and resuspended in RPMI 1640 medium supplemented with 10 percent fetal calf serum to test in vitro tumoricidal activity, as previously reported. D275-stained K562 cells (a particular target for NK cells) were killed at a 40:1 effector: target ratio using an assay technique established in our laboratory for evaluating cytotoxicity. Flow cytometry was used to measure the proportion of target cells that were destroyed using propidium iodide exclusion.

Statistical Analysis

The analysis was carried out using SPSS 26.0. Repertoire Using Dunnett's post hoc tests using week six, analysis of variance revealed major differences concerning time points 3 and all subsequent time points. A p-value of at least 0.05 was necessary to reject null hypothesis of no treatment effect. The Wilcoxon rank sum test was performed to compare immunological responses to *Bifidobacterium lactis*.

Results

Clinical Observations

The low-dose group had one subject withdraw before the intervention because they did not enjoy the taste of reconstituted milk and were experiencing some stomach pain, out of the original 400 participants. After the participant withdrew from the study, follow-up research found that these symptoms disappeared. The diets were followed by every other participant, according to their reports. Probiotic supplemented milk did not cause any additional adverse health impacts or general health issues over the nine-week trial period, as the individuals stated.

Overall Variations in Immune Variables

There were no significant changes in overall immunological characteristics amongst the typical-dose and low-dose groups in between-group analyses. However, both groups showed substantial time-dependent effects from therapy. Between the first and second snapshots, not much had changed. Some immunological factors changed significantly between the first and second time points (before intervention) and the third time point (postintervention). CD4–MHC II–restricted T cells, T lymphocytes, CD56+ NK cells and CD25–interleukin 2 receptor–positive T lymphocytes, increased considerably following ingestion of *Bifidobacterium lactis (*Table 1). Table 1 shows that these values decreased following the three-week washout interval without probiotic administration (although this fall was only significant in the case of CD25+ cells as compared to time point 3). The percentages of CD8+, CD19+, and HLA-DR+ stained cells did not change during the course of the study. Following the ingestion of B. lactis, both polymorphonuclear and mononuclear cells showed enhanced phagocytic activity in vitro. *Bifidobacterium lactis* consumption also resulted in a considerable increase in K562 cell tumoricidal activity. There was a substantial difference in in vitro cellular function amongst time points I, II, and IV in all instances (Table 1).

Table I. Partially stained mononuclear leukocytes at each of four time points in the first trial of probiotic supplementation

	Trials time point			
	I (standard)	II (after three weeks of non-supplemented LFM)	III (three-week after supplementation with *Bifidobacterium lactis*)	IV (three week after cessation of *Bifidobacterium lactis*)
B cells CD19+	11.9 ± 1.5	10.2± 1.4	12.3 ± 1.4	9.6 ± 1.4
APCs HLA-DR+	14.7 ± 1.5	16.3 ± 1.4	16.2 ± 1.4	14.7± 1.2
T-cells Total (CD3+)	64.1 ± 2.2	64.9 ± 2.7	66.9 ± 2.5	64.9 ± 2.8
CD25+	6.5 ± 0.7	6.8 ± 0.7	11.0 ± 1.0	7.5 ± 0.7
CD8+	18.2 ± 2.2	20.4 ± 2.0	19.7 ± 2.1	19.6 ± 2.1
CD4+	42.0 ± 1.7	41.9 ± 1.8	46.2 ± 1.4	44.6 ± 1.9
Natural killer cells CD56+	13.7 ± 1.3	14.8 ± 1.5	17.3 ± 1.5	15.8 ± 1.7

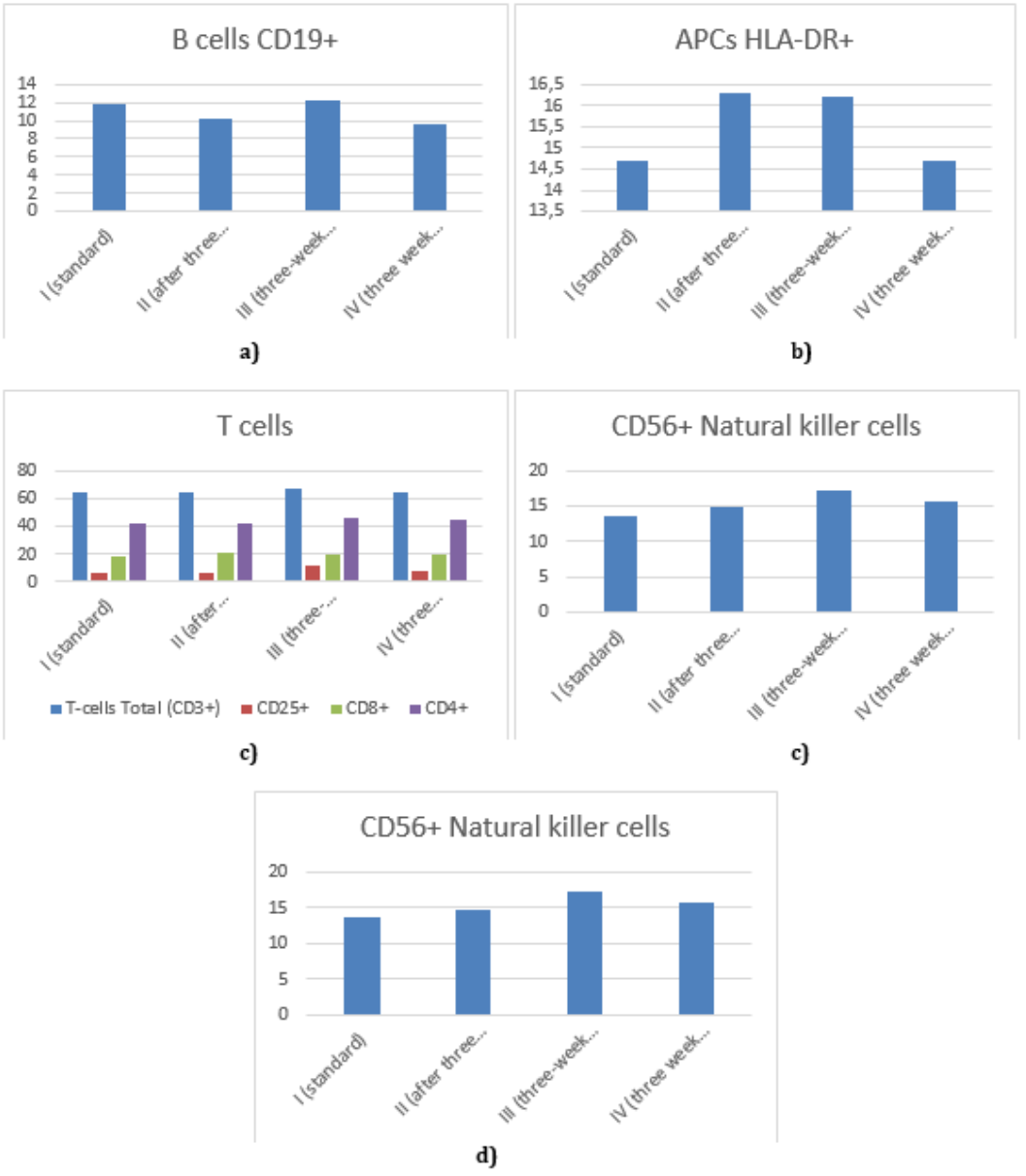

Figure 1. Partially stained mononuclear leukocytes at 1 to 4 time points in the probiotic supplementation trial 1

Improvements In Cellular Immune Function With *Bifidobacterium Lactis* (Probiotics)

As the immune responses increased between time points II and III, we could more carefully examine the phagocytic and tumoricidal activities of mononuclear and polymorphonuclear cells. Between 14.5 percent and 61.8 percent of the cellular

immune function was increased by the use of *B. lactis*, with the largest impact on NK cell activity (Table 2). Despite the fact that gains in the low-dose group were larger, there were no significant changes in the proportional magnitude of immunological enhancement amongst the typical-dose and low-dose groups. As shown in Figure 2, the preintervention immunological responses of the subjects were categorized into two categories: weak and sufficient. Between time points 2 and 3, the percentage changes in the immune responses of the poor and sufficient groups were compared. Immune function in individuals with inadequate preintervention immunity increased more than it did in those with good preintervention immunity on a consistent basis (Table 2). In the typical-dose group, these changes were considerable for phagocytosis of mononuclear and polymorphonuclear cells.

Table II. *Bifidobacterium lactis* consumption increased the percentage of patients' in vitro immune responses from time point 2 to time point 3 in both dosing groups

Variables of the immune system and the dose group 2	Participants included	Subjects who have a sufficient level of immunity prior to the procedure (%)	Subjects having a low level of immunity prior to intervention (%)
NK cell tumoricidal activity			
Low dose	61.8 (23.8, 99.8)	48	81
Typical dose	52.1 (17.4, 86.8)	36	67
Mononuclear cell phagocytosis			
Low dose	40.3 (20.8, 59.8)	29	57
Typical dose	38.2 (20.2, 56.2)	24	52
PMN cell phagocytosis			
Low dose	18.5 (9.5, 27.5)	17	23
Typical dose	14.5 (7.6, 21.4)	10	23

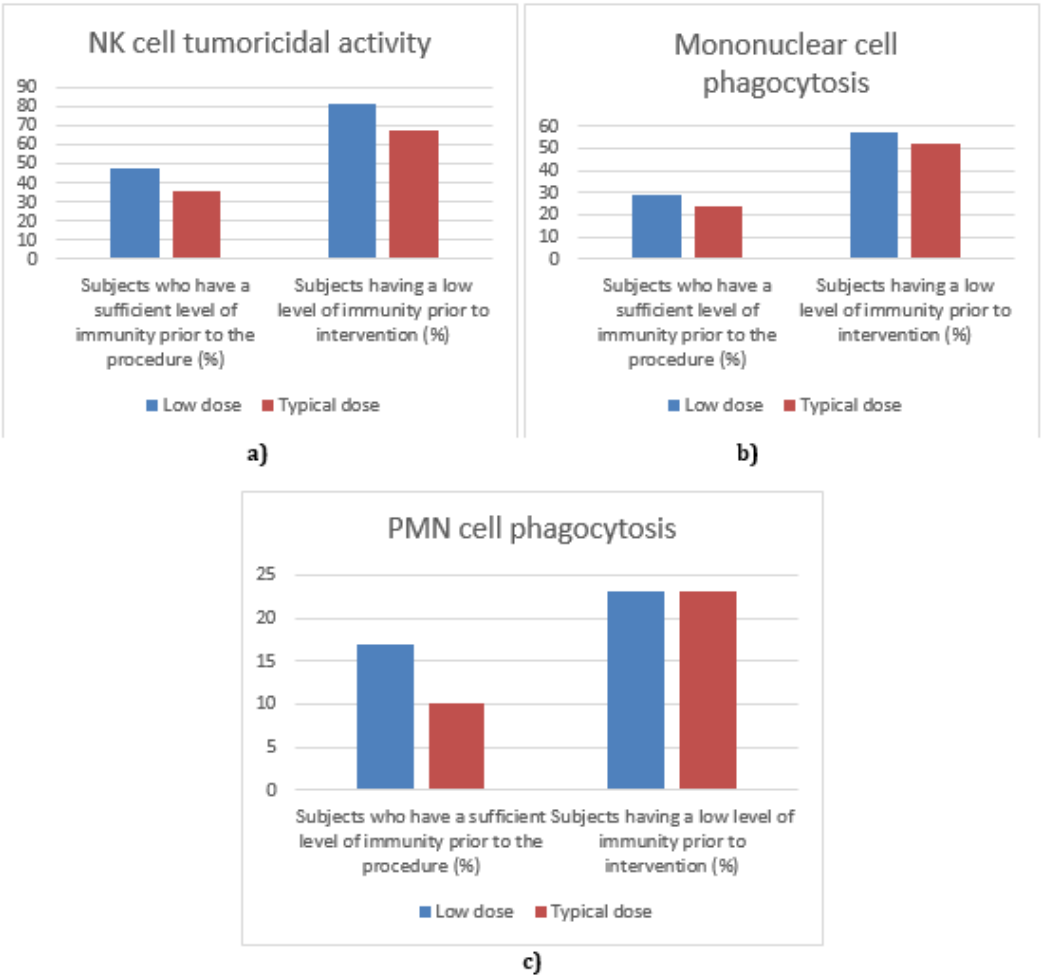

Figure 2. Invitro response of subjects after consumption of *B. lactis* from time point 2 to 3

Discussion

This study's findings imply that supplementing the elderly with an immunostimulant probiotic for three weeks may alter some features of their cellular immunity. Dietary intervention has already been postulated as a potential strategy of combating immunosenescence, and this may have practical importance for improving immunological function in children. Nutritional supplements have been shown to increase T lymphocyte subsets and enhance Natural killer cell activity in the elderly in prior research.[18] Nutritional therapies may be an effective way to enhance health outcomes that are dependent on an efficiently functioning immune system, according to one study. Reduced proportions of each of these lymphocyte phenotypes indicate immunosenescence. The current research found that supplementing children's diets with *Bifidobacterium lactis* led to small increases in immune cell populations, which are consistent with prior investigations of micronutrient supplementation.

Patients who received multivitamin and mineral supplements had a rise in circulating lymphoid cells, which correlated with increased antibody responses to influenza vaccination and a considerable reduction in infection-related morbidity. In addition, supplementation of *Bifidobacterium lactis* improved cellular immunological function, tumoricidal, and leukocyte phagocytosis activity in the body. However, it is not clear that the increases in tumor-killing activity was due completely or in part to higher blood NK cell ratios. Protection against bacterial infection and the growth of tumors may be improved by *B. lactis'* phagocytic and tumoricidal responses, and this is particularly true for geriatric health, where both illnesses are of particular concern. Adults who took certain strains of probiotic bacteria showed improved natural immunity (i.e., immunity not mediated by lymphocytes).[19] The Shirota strain of *Lactobacillus casei* and *L. rhamnosus* GG have both been demonstrated to improve immunity and prevent tumor development in adults as well as children, however it is unclear whether these physiological processes were the primary result of improved immunity. *B. lactis* intake has not been shown to raise disease resistance in the elderly or children, although it may do so via increasing innate cellular immune responses. *B. lactis* ingestion has the highest potential benefit for those with weak immune systems, according to the findings of Table 2. In this way, *B. lactis* may be the most promising candidate for immune-enhancing dietary supplements in terms of improving or restoring effective immunity.[20]

A modest dosage of *B. lactis* was shown to be effective in boosting the immunological response in this investigation. Typical dosages of 1×10^{10} to 1×10^{11} probiotic lactic acid bacteria species per day were shown in previous research to have physiological effects but dosages of less than 1×10^{9} organisms were not. A dosage of *B. lactis* (4×10^{9} organisms/d) consumed in this trial increased cellular immunity in a manner similar to that shown earlier with a 10-fold larger dose.[21] *B. lactis* was consumed continuously for three weeks with no adverse responses, corroborating earlier research showing that this strain is biologically harmless.[22] Thus, the safe dietary supplement *B. lactis*(probiotics) may be an effective way to boost the body's natural immune system and counteract some of the detrimental consequences of immunosenescence.

Conclusion

The present study concluded that supplementing the diets of children with *B. lactis* (a probiotic) may help improve their cellular immunity and physical development. From the above result substantial difference in their variables of immune system was observed. The results of this research proved *B. lactis* (probiotics) to be one of the promising dietary supplements and can be used to treat children who are under care of pediatricians.

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